

Review Article

Lipids and Cardiovascular Organ Damage in Type 2 Diabetes Mellitus

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Abstract

One of the mechanisms underlying increased cardiovascular (CV) risk in patients with type 2 diabetes mellitus is atherogenic dyslipidemia, that is characterized by elevated triglycerides and free fatty acids (FFAs) levels, low levels of high-density lipoprotein cholesterol (HDL) and an excess of small dense low-density lipoprotein particles (sdLDLs). Each component of atherogenic dyslipidemia is associated with CV events and triggers alterations at different levels of CV system through different pathways. FFAs and sdLDLs induce endothelial dysfunction, intima-media thickening, plaque formation and arterial stiffening through increase in oxidative stress and inflammation and promoting lipid

accumulation and smooth muscle cells (SMCs) proliferation in vascular wall. In contrast, HDL exerts protective effect on arterial wall by increasing nitric oxide availability, by reverse cholesterol transport and by suppression of SMCs proliferation and migration. FFAs overload results in a switch in myocardial substrate utilization, causing changes in myocardial energy metabolism and an increase in baseline oxygen consumption. Accumulation of toxic lipid intermediates in myocardium provokes damage of cellular membrane integrity, organelle dysfunction and apoptosis with consequent decrease in myocardial performance. The structural and functional changes in myocardium can be reversed by therapy with reconstructed HDL. Therefore, the impact of

atherogenic dyslipidemia on CV system is not limited on accelerated atherosclerosis, but causes different organ damages that must be considered in their complexity.

Keywords: Type 2 diabetes mellitus; Dyslipidemia; Endothelial function; Intima-media thickness; Arterial stiffness; Left ventricular remodeling

Introduction

The risk of cardiovascular (CV) disease in patients with type 2 diabetes mellitus (T2DM) is increased approximately 2-fold in men and 3-4-fold in women [1]. One of the mechanisms underlying such an increase is diabetic atherogenic dyslipidemia that is characterized by a cluster of interrelated lipids abnormalities, including hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol and the predominance of small dense low-density lipoproteins (sdLDLs) [2].

Altered metabolism of triglyceride-rich lipoproteins is critical in the development of the diabetic dyslipidemia. Increased flow of free fatty acids (FFAs) and glucose to the liver in the presence of insulin resistance results in increased secretion of large triglycerides-rich very low-density lipoproteins (VLDLs-1) [3-4]. VLDLs-1 secretion depends mainly on substrates availability but may be increased also by other T2DM-related factors, like inflammatory state and low adiponectin concentration. Overproduction of VLDLs-1 particles promotes the generation of sdLDL particles and reduces HDL levels. SdLDLs are more atherogenic than normal-sized LDL particles due to their longer plasma half-life and weaker resistance to oxidative stress [5]. Reduction in HDL levels is a

consequence of the exchange of triglycerides and cholesteryl ester between HDL and triglyceride-rich lipoproteins and formation of triglyceride-rich HDL that are quickly cleared from plasma by the action of hepatic lipase [4, 6].

T2DM is a chronic low-grade inflammation state and inflammatory cytokines influence plasma lipid levels. Interleukins (IL) and tumor necrosis factor (TNF) stimulate de novo synthesis of triglyceride-rich lipoproteins in liver and ILs also delay their clearance by decreasing lipoprotein lipase activity [7]. ILs and TNF- α decrease the production of apolipoprotein A-I (apoA-I) and IL-6 enhances its degradation by up-regulating the expression and activity of matrix-metalloproteinases (MMPs) [8-10]. In addition, inflammation induces changes in HDL structure that reduce their ability of reverse cholesterol transport as well as their anti-oxidant action [11].

Each component of atherogenic dyslipidemia is associated with CV events. In meta-analyses of prospective studies, for 1-mmol/L increase in plasma triglycerides there was a 32% increase in coronary disease risk for men and 76% increase in risk for women [12], and for 1-SD increment in HDL concentration, there was a 18% reduction in the risk of CV events [13], independently of other lipid fractions. In a general population without a history of CV disease, for 10 mg/dL increase in sdLDLs there was a 21% increase in CV disease [14], and in a large prospective cohort of subjects undergoing coronary angiography, an adjusted HR of the fourth FFA quartile for death from CV causes was 1.83 [15].

A strong association between atherogenic dyslipidemia and CV risk reflects the negative impact of lipids on CV system. Well-known is the role of lipid abnormalities in atherogenesis, yet, FFAs, sdLDLs and decreased or altered HDL may trigger adverse changes at different levels of CV system and thus cause

different organ damages. Complex inter-relationships between lipids and inflammation induce endothelial dysfunction, increase in carotid intima-media thickness (CIMT) and plaque formation, arterial stiffening and left ventricular (LV) remodeling and dysfunction (Table 1).

	Endothelial Function	IMT and Plaques	Arterial Stiffness	LV Remodeling and Dysfunction
FFAs	<p>↓</p> <p>↑ oxidative stress</p> <p>↑ inflammation</p> <p>↑ apoptosis of ECs & EPCs</p>	<p>↑</p> <p>↑ VSMCs proliferation & migration</p> <p>↑ ECM formation</p> <p>↑ inflammation</p> <p>↑ oxidative stress</p>	<p>↑</p> <p>↓ NO availability</p> <p>↑ inflammation → MMPs-activation</p>	<p>↓ energetic efficiency</p> <p>↑ oxidative stress</p> <p>↑ apoptosis</p> <p>↑ inflammation</p>
sdLDLs	<p>↓</p> <p>↑ oxidative stress</p>	<p>↑</p> <p>↑ lipid accumulation</p> <p>↑ inflammation</p>	<p>↑</p> <p>↓ NO availability</p> <p>↑ inflammation → MMPs-activation</p>	
HDL	<p>↑</p> <p>↑ eNOS activation</p> <p>↓ inflammation</p> <p>↓ apoptosis</p> <p>cholesterol efflux from ECs</p>	<p>↓</p> <p>reverse cholesterol transport</p> <p>↓ VSMCs proliferation & migration</p>	<p>↓</p> <p>↑ eNOS activation</p> <p>↓ inflammation</p> <p>positive impact on VSMCs phenotype & ECM expression</p>	<p>↓ inflammation</p> <p>↓ oxidative stress</p>

Table 1: Lipids and Cardiovascular Biomarker

Legend: EPCs: endothelial progenitor cells; ECs: endothelial cells; eNOS: endothelial nitric oxide synthase; NO: nitric oxide; VSMCs: vascular smooth muscle cells; ECM: extracellular matrix; LV: left ventricular; MMPs: mMatrix-metalloproteinases

All these alterations are established biomarkers of CV risk as numerous prospective studies have demonstrated their independent association with CV events. A 1% increase in brachial flow-mediated dilation decreases the risk of CV events by 13% [16], 1-SD increase in CIMT increases the risk of myocardial infarction and stroke by 26% and 32%, respectively [17], 1-SD increase in carotid-femoral pulse-wave velocity increases the risk of CV events by 47% [18], the presence of LV hypertrophy increases the risk of major CV events by 53% [19], and a 10% decrease in LV ejection fraction below 45% increases the risk of all-cause mortality by 39% [20].

Endothelial Dysfunction

Endothelium is the major regulator of vascular homeostasis, since it maintains the balance between vasodilation and vasoconstriction, inhibition and stimulation SMCs proliferation and migration, thrombogenesis and fibrinolysis. Accordingly, endothelial dysfunction is characterized by a shift of endothelial actions towards reduced vasodilation, a proinflammatory and prothrombic state. Mechanisms that participate on endothelial dysfunction include diminished production and/or availability of nitric oxide (NO), oxidative stress, endoplasmic reticulum stress, metabolic stress and inflammation [21].

Lipoprotein lipase located on the endothelial surface or within the arterial intima hydrolyzes triglycerides into FFAs and monoacylglycerols [22]. FFAs activate transcription factors that trigger oxidative stress and inflammation in the endothelium, facilitate apoptosis of endothelial cells and endothelial progenitor cells [23-25]. FFAs increase oxidative stress by generating reactive oxygen species (ROS) through protein kinase

C-dependent activation of NADPH oxidase [26], by increased production of superoxide (O_2^-) in mitochondrial respiratory chain [27] and by decreased NO production due to attenuation of endothelial Ca^{2+} signaling [28]. FFAs induce inflammation by activation of TNF- α , IL1- β , and IL-6 through the nuclear factor- κ B (NF- κ B) pathway [29].

Experimental studies demonstrated that a short-term exposure of endothelial cells to FFAs inhibits endothelial nitric oxide synthase (eNOS) activity and increases O_2^- release [24], while a long-term exposure triggers apoptosis and reduces total eNOS levels [25]. Clinical studies in healthy volunteers confirm the impact of FFA on endothelial function. The interventions leading to acute increase of plasma FFA, like ingestion of a single high-fat meal or intralipid infusion, impair endothelium-dependent vasodilation, but do not affect the flow response to nitroprusside [30-31].

SdLDLs decrease production of cytoprotective NO and increase production of cytotoxic peroxynitrite ($ONOO^-$) in endothelial cells [32]. In healthy men, LDL size has been shown to correlate with endothelium dependent vasodilation; men with small LDL particles had a 39% lower blood flow response to acetylcholine than men with large LDL particles, independently of LDL cholesterol concentrations [33]. In T2DM patients, LDL size predicted the forearm blood flow response to acetylcholine, independently of age, gender and blood pressure [34].

HDL exerts protective effect on endothelium by activating endothelial nitric oxide synthase (eNOS) and by reducing inflammation, apoptosis and

thrombosis. HDL particles, above all apoA-I, activate protein kinase Akt and eNOS through interactions with receptors present on endothelial cells (scavenger receptor class B, type 1 and sphingosine-1-phosphate receptor) [35-36]. HDL also inhibits TNF- α -dependent NF- κ B activation [37] and mediates cholesterol efflux from endothelial cells that decreases the cholesterol content of caveolae, reduces inhibitory interaction of eNOS with caveolin-1 and promotes NO synthesis [38]. The positive impact of HDL on endothelial function was demonstrated in hypercholesterolemic men with reduced endothelium-dependent response of forearm blood flow to acetylcholine and preserved endothelium-independent response to sodium nitroprusside. An intravenous infusion of reconstructed HDL increased plasma HDL levels and enhanced the acetylcholine-induced increase in forearm blood flow without affecting the response to sodium nitroprusside [39].

Carotid Intima-Media Thickening and Plaques

Increase in carotid intima-media thickness (CIMT), which represents a combined measure of intimal and medial thicknesses, is considered a very early marker of subclinical atherosclerosis or adverse arterial remodeling, while the presence of carotid plaques reflects more advanced atherosclerotic lesion.

FFAs may alter CIMT by several mechanisms. At the medial layer, FFAs stimulate vascular SMCs proliferation and migration and modify the expression of genes controlling extracellular matrix formation. At the intimal level, FFAs induce oxidative stress, apoptosis, and an inflammatory response. An independent association between circulating FFAs and CIMT was demonstrated in healthy men [40] as well

as in renal transplant recipients [41]. In T2DM patients, FFA-rich areas were found within carotid plaques, and the pattern of distribution of plaque FFAs was similar to that of monocyte chemoattractant protein-1 and activated NF- κ B, i.e. to that of inflammation [42].

Lipid accumulation in the arterial wall is a crucial event in the development of atherosclerotic lesions. While the native LDLs does not trigger lipid accumulation, modified particles, like oxidized and glycated LDLs, are highly atherogenic and possess proinflammatory properties. SdLDLs have a higher susceptibility to lipid peroxidative modification, when compared to larger LDLs, which can be explained by their composition (lower lipid and higher apoB content) [43] and by lower concentration of antioxidants [44]. SdLDLs are also more susceptible to glycation since apoB is preferentially glycosylated in sdLDL particles [45]. Higher glycation and oxidation of sdLDLs are accountable for increased atherogenic potential. In healthy men, plasma sdLDLs were directly and independently associated with CIMT [46], in patients with T2DM and prediabetes, the proportion of sdLDL particles at baseline predicted CIMT increase at 2 years [47], and in patients with acute cerebral infarction, sdLDLs were an independent risk factor for unstable carotid plaques [48].

The most important mechanism by which HDL prevents the initiation and progression of atherosclerotic changes is a reverse cholesterol transport. HDL scavenges cholesterol from the peripheral vasculature and transports it to the liver, where it is excreted in the biliary system. Cholesterol is removed from the monocyte macrophage system by

passive diffusion, by facilitated diffusion mediated by scavenger receptor class B, type 1, or by active pathways mediated by the ATP binding cassette transporters [49].

Experimental studies in animals have demonstrated a reduction in plaque size, lipid, and macrophage content with infusions of either human HDL or reconstituted HDL containing apoA-I [50]. In humans, intravenous infusions of reconstructed HDL containing wild-type apoA-I did not reduce the volume of coronary plaques but had favorable effect on a plaque echogenicity index consistent with potential plaque stabilization [51]. This is in agreement with studies on carotid arteries, showing that low HDL levels are associated with echolucent, rupture-prone plaques [52]. Different studies also demonstrated an inverse association between HDL and CIMT [53], however, these associations probably reflect HDL-induced suppression of SMCs proliferation and migration rather than a reverse cholesterol transport [54].

Arterial Stiffness

Arterial stiffness depends mainly on the relative content of 2 structural proteins, elastin and collagen. Disarrangement and fragmentation of elastin fibers together with overproduction and cross-linking of collagen lead to arterial stiffening. In T2DM patients, arterial stiffness is increased across all age groups and one of the main mechanisms involved is the formation of advanced glycation end-products (AGEs), causing cross-linking of collagen molecules. On arterial stiffening in diabetes may participate also hyperinsulinemia- and hyperglycemia-induced increase in the local activity of the renin-angiotensin-

aldosterone system that promotes arterial wall hypertrophy and fibrosis.

The role of lipids in arterial stiffening is mediated by endothelial dysfunction and inflammation. NO, whose reduced bioavailability represents the molecular basis of endothelial dysfunction, is involved also in the regulation of arterial distensibility, and NO donors have been shown to reduce augmentation index and wave reflection, independently of their effect on blood pressure. Systemic and local inflammation trigger arterial stiffening through cytokines that induce transcription and activation of MMPs. MMPs cleave the protein components of the extracellular matrix, including elastin, and thereby play a central role in vascular remodeling and stiffening. Numerous cross-sectional and prospective clinical studies demonstrated the independent association of arterial stiffness indices with triglycerides, FFAs and sdLDL (55-57) as well as with cytokines and MMPs, not only in T2DM patients but also in general population, healthy subjects, patients with hypertension or chronic inflammatory disease [54, 58-59].

In contrast to FFAs and sdLDL, HDL can prevent arterial stiffening. ATP binding cassette transporter A1 (ABCA-1), one of the cell membrane proteins participating in the cholesterol efflux from cells to apolipoproteins, has been shown to modulate vascular SMCs phenotypic switch and to suppress the expression of inflammatory cytokines that may activate MMPs [60-61]. In addition, apolipoprotein E (apoE) and apoE-containing HDL suppress extracellular matrix gene expression in response to mechanical stimuli [62] and upregulate ABCA-1 expression [63]. Indeed, in healthy subjects, aortic

stiffness was inversely related to ABCA-1-dependent serum cholesterol efflux capacity [64], and ApoE-deficient mice showed increased aortic stiffness and focal fragmentation of elastic fibers in aortic wall [65].

LV Remodeling and Dysfunction (Diabetic Cardiomyopathy)

The heart is the organ with the highest oxygen consumption rate per weight unit. For adenosine triphosphate (ATP) production, the heart can utilize different substrates in order to maintain consistent ATP production with changing substrate availability. In normal adult heart, ~60-90% of the acetyl-CoA comes from β -oxidation of fatty acids, and 10-40% from the oxidation of pyruvate that is derived in approximately equal amounts from glycolysis and lactate oxidation. In diabetic heart, there is a shift towards FFAs utilization. This could be explained by the fact that the rate of FFAs uptake by myocardium is not hormonally regulated but largely driven by FFAs plasma concentration, while glucose uptake is regulated by insulin. Combination of insulin resistance and FFA overload favors FFA utilization. In addition, increased FFAs flow stimulates cardiac peroxisome proliferator-activated receptor- α (PPAR- α) that up-regulates the lipid metabolic pathway while the products of mitochondrial FFA oxidation repress cellular glucose utilization through allosteric inhibition of glycolytic enzymes [66]. As a result of all these changes, diabetic myocardium depends almost entirely on FFAs as the energy source [67]. Substrate switch from glucose to FFAs reduces the efficiency of oxidative phosphorylation since every molecule of ATP generated from the oxidation of FFA costs 0.3 molecules of oxygen more than ATP generated from glucose [68]. Indeed, experimental studies in animals

demonstrated that diabetic hearts consume $\geq 30\%$ more oxygen than non-diabetic hearts, while generating the same contractile force [69].

Seventy-ninety percent of FFAs taken up by the myocardium is rapidly utilized for energy metabolism in the mitochondria while 10-30% is stored as triacylglycerol to be rapidly mobilized in conditions of increased myocardial energy demands. When the capacity for mitochondrial fatty acid β -oxidation cannot keep up with the excessive FFAs delivery, a number of various toxic lipid intermediates, particularly diacylglycerols and ceramides, begins to accumulate in myocardium inducing oxidative stress that results in damage of cellular membrane integrity, organelle dysfunction and apoptosis [70]. In animal models, obese Zucker diabetic rats showed elevated intramyocardial accumulation of triglycerides and ceramide, increased apoptosis and LV dilatation with reduced contractility. Suppression of plasma triglyceride with the PPAR γ agonist troglitazone lowers myocardial triglyceride and ceramide content, prevent apoptosis and deterioration of cardiac function [71].

Corresponding findings were observed in human heart using proton magnetic resonance spectroscopy. In a study comparing lean normoglycemic subjects, subjects with impaired glucose tolerance and diabetic subjects, myocardial triglyceride content was 2.3-fold and 2.1-fold higher in subjects with impaired glucose tolerance and T2DM, respectively, as compared to lean normoglycemic subjects. LV systolic function was preserved in all 3 groups, yet, the estimate of diastolic function was abnormal in impaired glucose tolerance and T2DM groups [72]. Acute

pharmacologic inhibition of adipose tissue lipolysis by a nicotinic acid analogue in diabetic patients resulted in reduction of FFAs plasma levels and myocardial lipids content by 69% and 39%, respectively, and in increase of ejection fraction by 15%. Changes in plasma FFAs concentration strongly correlated with changes in myocardial lipid content and with ejection fraction ($r=0.71$ and 0.65) [73].

Besides altered energy metabolism and intramyocardial lipid accumulation, diabetic cardiomyopathy is characterized by myocardial inflammation and fibrosis. Several molecular mechanisms may be involved in diabetes-related myocardial inflammation, yet majority of these mechanisms converge towards the activation of the NF- κ B pathway, with consequent upregulation of cytokines, chemokines and adhesion molecules, that can be attenuated by PPAR- β/δ [74]. TNF- α , IL-6 and IL-1 β directly promote cardiomyocyte growth and hypertrophy as well as contractile dysfunction [75-76]. TNF- α and IL-6 also stimulate cardiac fibroblast proliferation and collagen production, whereas IL-1 β activates MMPs [77-78], all of which generate myocardial fibrosis.

HDL and apoA-I have important anti-inflammatory potential. They can remove active TNF- α , inhibit the production of IL-1 β and TNF- α , induce the expression of anti-inflammatory cytokine IL-10 and reduce expression of VCAM-1, ICAM-1 and E-selectin [79]. Several experimental studies investigated the anti-inflammatory impact of HDL on diabetic heart. In streptozotocin-induced rat model of diabetes, apoA-I gene transfer increased HDL levels, decreased cell adhesion molecules and TNF- α expression and

decreased total collagen content in myocardium [80]. Mice fed with a high-sugar/high-fat diet developed cardiac hypertrophy with increased interstitial and perivascular fibrosis and impaired systo-diastolic function. Treatment with reconstituted HDL_{Milano} reversed pathological remodelling and cardiac dysfunction [81].

In clinical studies, LV mass and relative wall thickness were inversely related to HDL in general population and in hypertensive subjects [82-83]. LV systolic function, assessed as ejection fraction or as a velocity of LV longitudinal shortening, and LV diastolic function, assessed as the longitudinal velocity of early diastolic filling, correlated with HDL, both in diabetic patients and non-diabetic subjects [53, 84-86].

Conclusions

The prevalence of diabetic dyslipidemia is high and each of its components is able to provoke deleterious changes at different levels of CV system (Table 1). Common perception of CV risk in T2DM patients is associated above all with accelerated atherosclerotic process, yet diabetes-related metabolic abnormalities, and lipid abnormalities in particular, had much wider negative effect on CV system as we try to summarize in this paper. It must be considered that the adverse impact of diabetic dyslipidemia on different parts of the CV system should be evaluated in its complexity. Diabetic patients, whose heart has impaired systole-diastolic performance and consummate 30% more oxygen at resting conditions, and whose afterload and therefore workload is increased due to arterial stiffening, can be expected to develop myocardial ischemia or heart failure with a lower degree of coronary stenosis or at a lower hemodynamic load than

non-diabetic subjects. Therefore, it is of utmost importance to understand the complex mechanism through which lipids may alter CV structure and function so that their negative impact can be detected in preclinical phase by non-invasive imaging techniques (ultrasound, magnetic resonance imaging, positron emission tomography) and mitigated by appropriate life-style and pharmacologic interventions [87].

Conflict of Interest

No conflict of interest to be declared.

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